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Vesicular-Arbuscular Mycorrhizal Colonization and Growth of Tomato *(Lycopersicon esculentum)* in Autoclaved Soil

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ABSTRAK

Kesan tanah autoklava terhadap pengkolonian kulat vesikal-arbuskular mikoriza (VAM) dan pengeluaran tomato biojisim telah dinilai dalam bekas yang diuji di rumah hijau. Empat rawatan telah diberi iaitu (1) Tumbuhan bebas-VAM dalam tanah autoklava (2) Tumbuhan VAM dalam tanah autoklava (3) Tumbuhan VAM dalam tanah autoklava yang diubah suai dengan tanah tanpa autoklava filtrat bebas-VAM (4) Tumbuhan VAM dalam tanah tanpa autoklava. Tumbuhan VAM yang membesar dalam tanah tanpa autoklava menunjukkan pengkolonian akar paling tinggi iaitu 87.78%, manakala (2) dan (3) masing-masing hanya 55.11% dan 56.94%. Di samping itu, panjang tunas (105.4 cm/tanaman), jumlah ruang daun (740.3 cm²/tanaman) dan biojisim (8.43 g/tanaman) diperolehi dalam tumbuh-tumbuhan VAM yang membesar dalam tanah autoklava. Tumbuhan bebas VAM dalam tanah autoklava kurang membesar. Dalam rawatan (3) dan (4) pembesaran tumbuhan adalah sederhana. Keputusan menunjukkan bahawa pengkolonian VAM dan pengsporaan sesuai dalam keadaan semulajadi tetapi tumbuhan VAM dalam keadaan tanah autoklava menghasilkan pertumbuhan yang maksimum.

ABSTRACT

*The effect of autoclaving soil on vesicular-arbuscular mycorrhizal (VAM) fungal colonization and biomass production of tomato (*Lycopersicon esculentum *Mill.) was assessed in pot experiments under greenhouse conditions. Four treatments were given viz., (1) VAM-free plants in autoclaved soil, (2) VAM plants in autoclaved soil, (3) VAM plants in autoclaved soil amended with VAM-free filtrate of non-autoclaved soil, and (4) VAM plants in non-autoclaved soil. The VAM plants grown in non-autoclaved soil showed the highest root colonization of 87.78% while those under (2) and (3) showed only 55.11 % and 56.94% respectively. On the other hand, significantly higher shoot length (105.4 cm/plant), total leaf area (740.3 cm²/plant) and biomass (8.43 g/plant) were obtained in VAM plants grown in autoclaved soil. VAM-free plants in autoclaved soil had reduced growth. In treatments 3 and 4 plant growth was intermediate. The results indicate that VAM colonization and sporulation were favoured under natural conditions, but VAM plants under autoclaved soil conditions produced maximum growth.*

INTRODUCTION

Partial or complete sterilization of soil often changes its nutrient status and structure (Lopes and Wollum, 1976; Mulder, 1979; Jakobsen and Andersen, 1982). Sterilization also removes some or all of the microorganisms (Bowen and Rovira, 1969). However, enhanced fertility due to autoclaving is available to plants only during their initial stage of growth.

Mosse *et al.* (1969) observed that soil sterilization had a positive effect on the development of vesicular-arbuscular mycorrhizal (VAM) infection and plant growth, but Wilson (1984) reported that autoclaving of soil inhibited VAM spore germination. Little is known about the effect of autoclaving of soil on VAM fungal colonization and biomass production in tomato (*Lycopersicon esculentum* Mill.) which this study attempted to elucidate.

MATERIALS AND METHODS

Preparation of substrates and raising of seedlings

Moderately fertile sandy loam soil collected from the experimental fields of Bharathiar University, Coimbatore (pH 8.1, EC 0.1 m S cm^{-1}, N 10.5 mg kg^{-1}, P 1.7 mg kg^{-1}, K 38 mg kg^{-1}), mixed with sand at 1:1 proportion was used as the substrate for plant growth. The substrate was sterilized in cloth bundles in an autoclave at 1.5 kg sq cm^{-1} pressure (121 °C) for 1 hour each on three consecutive days and left in the laboratory for seven days to facilitate release of any toxic substances produced during heating. Ten presterilized pots of 18 cm diam. were filled with 6 kg pot^{-1} of autoclaved soil and another 30 pots were filled with non-autoclaved soil-sand mixture.

The field soil contained VAM fungal spores predominantly of *Acaulospora bireticulata* Rothwell & Trappe; A. *sporocarpia* Berch; *Glomus deserticola* Trappe, Bloss & Menge; *G. fasciculatum* (Thaxter & Gerdemann) Gerdemann & Trappe; *G. geosporum* (Nicolson & Gerdemann) Walker; *G. tenue* (Greenall) Hall and *G. sinosum* (Gerdemann & Bakshi Almeida & Schenck (= *Sclerocystis pakistanica* Iqbal & Bushra), having a total spore count of 20.43 (± 0.73) spores g^{-1} dry soil. The VAM fungal species were identified using synoptic keys (Hall, 1984; Morton 1988; Schenck and Perez, 1987) for spores and sporocarps.

All pots were sown with uniform sized seeds of tomato cv. Co. 1 at a density of 5 per pot and watered regularly. One week after germination, they were thinned to maintain one healthy seedling per pot and allowed to grow for 30 days under greenhouse conditions. VAM fungal infection was detected by the method of Philips and Hayman (1970) in the seedlings raised on non-autoclaved soil, while those from autoclaved soil were free from any infection.

Transplanting of seedlings

Two sets of potted soil, i.e. 30 pots of autoclaved and 10 non-autoclaved, were prepared as in the previous experiment. Of these, 10 pots of autoclaved soil were drenched with soil filtrate of non-autoclaved soil at the rate of 100 ml pot^{-1} to facilitate VAM and mycophagous animal free microbial action in the soil. The filtrate was prepared by adding 500 ml of sterilized water to 350 g of non-autoclaved soil and thoroughly mixing. The liquid portion of mixture was decanted and filtered through a 38 mm mesh that retained VAM fungal spores and mycophagous animals but not other microbes (Azcon-Aguilar and Barea, 1985).

The 30-day-old VAM-free tomato seedlings in the autoclaved potted soil were transplanted into 10 pots of autoclaved soil with one seedling per pot as Treatment 1, and out of the 30 VAM seedlings in the non-autoclaved potted soil, 10 pots each with one seedling were transplanted into autoclaved soil (Treatment 2), autoclaved soil amended with filtrate (Treatment 3), and non-autoclaved soil (Treatment 4). There were six replicates for each treatment.

All the transplanted seedlings under different treatments were allowed to grow for another 60 days (90 days in total) without adding any fertilizer, when they were harvested for estimation of VAM colonization, sporulation and growth parameters.

Laboratory analysis

Soil pH, EC and nutrient status were analysed using standard procedures (Misra, 1968; Jackson, 1973). For estimation of VAM colonization, at harvest, 0.20 g (wet weight) was excised from each root system. The rest of the root system was kept for observing dry weight. VAM colonization index (VAM1) was estimated after staining the root samples following the method of Phillips and Hayman(1970) and using the scoring method of Edathil *et al.* (1994). The number of VAM fungal spores of the rhizosphere soil of different treatment plants was estimated by wet-sieving and decanting method (Gerdemann and Nicolson, 1963). Spore counting was done with 100 g dry soil and spore density was expressed as number of spores per gram of soil.

The total leaf area was measured using a leaf area meter and the mean of six replicates of each treatment calculated.

Root length, shoot length, root dry weight, shoot dry weight, root/shoot (R/S) ratio for dry weight and mycorrhizal dependency were recorded. Dry weights were obtained by drying for 24 h at 45°C in a hot air oven. Mycorrhizal dependency (M.D.) was calculated using the formula of Plenchette *et al.* (1983):

$$\text{M.D.} = \frac{\text{(DM of VAM plant - DM of VAM-free plant)}}{\text{(DM of VAM-free plant)}} \times 100$$

where DM = dry mass.

RESULTS

The highest percentage of VAM colonization and spore density was observed in the VAM plants grown in non-autoclaved soil, while those in autoclaved soil (amended or non-amended with soil filtrate) showed significantly lower root colonization. However, the number of spores was significantly reduced only in the filtrate amended treatment (Table 1). Altogether 17 species of VAM fungi belonging to 3 genera were recorded from the rhizosphere soils of the VAM plants irrespective of treatment conditions (Table 2).

For plant growth, significantly higher ($P < 0.01$) shoot length, total leaf area and biomass were obtained in VAM plants grown in autoclaved soil while lowest growth was recorded in VAM-free plants grown in autoclaved soil. There was no marked difference in root length or R/S ratio among VAM plants and in autoclaved and non-autoclaved soils. However R/S ratios were higher in VAM-free plants and in VAM plants grown in the substrate amended with soil microbes (Table 3; Fig. 1).

The VAM-free plants in autoclaved soil showed good growth initially; they became stunted in due course, but retained a lush green colour unlike plants in non-autoclaved soil which had stunted growth and were pale by the end of the experiment.

TABLE 1
Effect of autoclaving of soil on the VAM fungal infectivity on tomato plants

Treatment	Per cent colonization index (PDI)	Spore counts g^{-1} soil
VAM-free plants in autoclaved soil	0	0
VAM plants in autoclaved soil	55.11[b]	11.54[a]
VAM plants in autoclaved soil amended with VAM-free filtrate of non-autoclaved soil	56.94[b]	9.51[b]
VAM plants in non-autoclaved soil	87.78[a]	12.39[a]
	P >0.01 CD 16.03	P >0.05 CD 1.83

Values with same alphabet in the same column are not significantly different.

TABLE 2
VAM fungal species recorded from the rhizosphere soil of VAM tomato plants after 90 days of growth

1. *Acaulospora bireticulata* Rothwell & Trappe
2. *A. trappei* Ames & Linderman
3. *Gigaspora albida* Schenck & Smith
4. *Glomus aggregatum* (Schenck & Smith) Koske
5. *G. deserticola* Trappe, Bloss & Menge
6. *G. fasciculatum* (Thaxter & Gerdemann) Gerdemann & Trappe
7. *G. fulvum* (Berk.) Pat.
8. *G. geosporum* (Nicolson & Gendermann) Walker
9. *G. intraradices* Schenck & Smith
10. *G. maculosum* Miller & Walker
11. *G. macrocarpum* (Tul. & Tul) Nicolson & Gerdemann
12. *G. manihotis* Howler, Sieverding & Schenck
13. *G. melanosporum* Gerdemann & Trappe
14. *G. microaggregatum* Koske, Gemma & Olexia
15. *G. mosseae* (Nicolson & Gerdemann) Gerdemann & Trappe
16. *G. tenue* (Greenall) Hall
17. *G. sinosum* (Gerdemann & Bakshi) Almeida & Schenck

P deficient soil. The suppressed growth of VAM plants with the addition of soil microbes (non-autoclaved soil filtrate) was also evident. VAM efficiency was mainly the result of the presence of extramatrical hyphae and the efficient VAM symbiosis, which reduced R/S ratio, and not the extent of per cent root colonization.

REFERENCES

AFEK, U., J.A. MENGE and E.L.V. JONHSON. 1990. Effect of *Pythium ultimum* and metalaxyl treatments on root length and mycorrhizal colonization of cotton, onion and pepper. *Plant Disease* **74:** 117-120.

ATILANO, R.A., J.R. RICH, H. FERRIS and J.A. MENGE. 1986. Effect of *Meloidogyne arenaria* on endomycorrhizal grape (*Vitis vinifera*) rootings. *Journal of Nematology* **8:** 278.

AZON-AGUILAR, C. and J.M. BAREA. 1985. Effect of soil microorganisms on formation of vesicular-arbuscular mycorrhizas. *Transactions of British Mycological Society* **84:** 535-537.

BENTIVENGA, S. 1988. *Effect of Inorganic Nutrient Treatment, Soil Microorganisms, and Vesicular-arbuscular Mycorrhizae on the Growth of Big Bluestem (*Andropogon gerardii*).* Master's Thesis, Illinois State University.

BOWEN, C.D. and A.D. ROVIRA. 1969. The influence of microoganisms on root growth and metabolism. In Wittington W.J. (ed) : *Root Growth.* London: Butterworths.

CLARKE, C. and B. MOSSE. 1981. Plant growth response to vesicular-arbuscular mycorrhiza. XII. Field inoculation responses of barley at two soil P levels. *New Phytologist* **87:** 695-703.

DANIELS, B.A. and J.A. MENGE. 1980. Hyperparasitization of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* **70:** 585.

DANIELS, B.A. and J.M. TRAPPE. 1980. Factors affecting germination of vesicular-arbuscular mycorrhizal fungus *Glomus epigaeum. Mycologia* **72:** 457-471.

DOUDS, D.D. and W.R. CHANEY. 1982. Correlation of fungal morphology and development to host growth in a green ash mycorrhiza. *New Phytologist* **92:** 519-526.

EDATHIL, T.T., S. MANIAN, and K. UDAIYAN. 1994. The effect of vesicular-arbuscular mycorrhizal exposure period on their colonization of and spore production in tomato seedlings *(Lycopersicon esculentum* Mill.*),* and on host biomass. *Agriculture, Ecosystems and Environment* **51**: 287-292.

FITTER, A.H. 1977. Influence of mycorrhizal infection on competition for phosphorus and potassium by the grasses. *New Phytologist* **79:** 119-125.

GERDEMANN, J.W. and T.H. NICOLSON. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving. *Transactions of British Mycological Society* **46:** 234-235.

GROTH, D.E. and C.A. MARTINSON. 1983. Increased endomycorrhizal infection in maize and soybeans after soil treatment and metalaxy. *Plant Disease* **67:** 1377-1378.

HALL, I.R. 1984. Taxonomy of VA mycorrhizal fungi. In Powell L.I., Bagyaraj D.J. (eds) *VA Mycorrhiza.* CRC Press Inc., Boca Raton, Florida. pp. 57-94.

HALL, I.R., P.D. JOHNSTONE and R. DOLLY. 1984. Interactions between endomycorrhizas and soil nitrogen and phosphorus on the growth of rye-grass. *New Phytologist* **97:** 447-453.

HERICK, B.A.D., D.G. KITT and G.T. WILSON. 1986. The influence of phosphorus fertilizer, drought, fungal species and non-sterile soil on the mycorrhizal growth response in tall grass prairie. *Canadian Journal of Botany* **64:** 1199-1203.

HERTRICK, B.A.D., G.T. WILSON, D.G. KITT and A. P. SCHWAB. 1988. Effect of soil microorganisms on mycorrhiza contribution to growth of big bluestem grass in non-sterile soil. *Soil Biology and Biochemistry* **20:** 501-507.

JACKSON, N.L. 1973. *Soil Chemcal Analysis.* Prentice Hall of India Pvt. Ltd. New Delhi.

JAKOBSEN, I. and A.J. ANDERSEN. 1982. Vesicular-arbuscular mycorrhiza and growth in barley. Effects of irradiation and heating of soil. *Soil Biology and Biochemistry* **14:** 171.

KHALIL, S., T.E. LOYNACHEN, and H.S. MC NABB JR. 1992. Colonization of soybean by mycorrhizal fungi and spore population in Iowa soils. *Agronomy Journal* **84:** 832-836.

LOPES, A.S. and A.G. WOLLUM. 1976. Comparative effectiveness of methylbromide, propylene oxide and autoclave sterilization on specific soil characteristics. *Turrialba* **26:** 351.

MEREDITH, J.A. and R.C. ANDERSON. 1992. The influence of varied microbial substrate conditions on the growth and mycorrhizal colonization of little bluestem. *New Phytologist* **121:** 235-242.

MISRA, R. 1968. *Ecology Work Book.* Oxford, IBH Publishing Co. Pvt. LTD., New Delhi.

MORTON, J.B. 1988. Taxonomy of VA mycorrhizal fungi: Classification, nomenclature and identification. *Mycotaxon* **32:** 267-324.

MOSSE, B., D.S. HAYMAN and G.J. IDE. 1969. Growth response of plants in unsterilized soil to inoculation with vesicular-arbuscular mycorrhiza. *Nature* (London) **224:** 1031-1032.

MULDER, D. (Ed.), 1979. *Soil Disinfestation: Some Scientific and Practical Contributions in the Field of Soil Disinfestation.* Elsevier: Amsterdam.

PHILLIPS, J.M. and D.S. HAYMAN. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society* **55:** 158-161.

PLENCHETTE, C., J.A. FORTIN and V. FURLAN. 1983. Growth response of several plant species to mycorrhizae in a soil of moderate P-fertility % mycorrhizal dependency under field conditions. *Plants and Soil* **70:** 199-209.

SCHENCK, N.C., R.A. KINLOCK and D.W. DICKSON. 1975. Interaction of endomycorrhizal fungi and root-knot nematode of soybean. In *Endomycorrhizas*, Sanders, Mosse, B. and Tinker, P.B. (Eds). Academic Press: London.

SCHENCK, N.C., and Y. PEREZ. 1987. *A Manual of Identification of Vesicular-arbuscular Mycorrhizal Fungi.* INVAM, University of Florida, Gainesville, Florida.

SEO, H.-A., R.C. ANDERSON and A.E. LIBERTA. 1988. Influence of varied soil microbial and inorganic nutrient conditions on the growth and VA colonization of little bluestem (*Schizachyrium scoparium*). *Biology and Fertility of Soils* **6:** 1-5.

WARNOCK, A.J., A.H. FITTER and M.B. USHER. 1982. The influence of a spring tail *Folsomia candida* (Insect, Collembola) on the mycorrhizal association of leek *Allium porrum* and the vesicular-arbuscular mycorrhizal endophyte *Glomus fasciculatum. New Phytologist* **90:** 285.

WILSON, J.M. 1984. Inhibition of germination of spores of a *Gigaspora* species in sterilized soils. *Soil Biology and Biochemistry* **16:** 433-435.

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